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Polyherbal formulation development and pharmacological screening

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ABSTRACT
Polyherbal formulation has been used all round the world because of its healthful and therapeutic application. It has also known as a polyherbal therapy or herb- herb combination.People are using herbal medicine from centuries for safety, efficacy, cultural acceptability and lesser effect. It is due to increase of awareness and knowledge about plants. Plant and plant product utilized with varying success to cure and disease throughout history. Therapeutically intresting and important drug can be develop from plant sources which are used in traditional system of medicine is based in empirical knowledge of observation and experience over millennia and more than 5000 plants are used in different ethic communities in India. In which curcuma longa, Zingiber officinalis, Ocimum sanctum , Solanum nigrum have large biodiversity in India.

Keywords: Curcuma longa, Solanum nigrum, Zingiber officinalis, Ocimum sanctum.

I. INTRODUCTION

God has gifted us with this beautiful nature which contain resourceful wild life. Herbal plant have great growth potential in global market.

Curcuma longa is a perennial herb and member of the Zingiberaceae (ginger) family and is cultivated in Asia mostly in India and china .The rhizome of the plant used medicinally, yields yellow powder.Dried Curcuma longa is the source of turmeric , the ingredient that gives curry powder it characterize yellow colour. Turmeric has been used in flavor and colour, and Chinese and Ayurvedic medicine particularly as an anti-inflammatory and for the treatment of jaundice, menstrual difficulties. It is official in the pharmacopeia of china as well as in ther Asian countriese such as japan and korea.Oral administration is the main route of administration for Curcuma longa. (1)

Ginger (Zingiber officinalis rocs.) belongs to the family Zingiberaceae. It originated in south–east Asia and then used in many countries as a spice and condiment to add flavor to food (2). Beside this, the rhizome of ginger has also been used in traditional herbal medicine. The health promoting perspective of ginger is attributed to its rich phytochemistry(3).It also has anti inflammatory and antioxidative propertiese for controlling the process of aging .

Tulsi may be extremely aromatic aromatic herb from the mint family that's endemic to the Indian landmass and been used inside Ayurvedic drug quite 3000 years.In the written material system tulsi is often reffered to as an elixier of life for its healing powers and has been known to treat many different common health condition.In the Indian material medica tulsi leaf extract are describe for treatment of bronchitis and pyrexia .(4)

Solanum nigrum is commonly known as "black night shade''belong to Solanaceae family.It is shows medicinal properties like anti–microbial, antioxidant, cytotoxic properties , anti –ulcerogenic activity.it is an African pediatric plant utilize for several alignments that are responsible for infant mortality especially feverish conversion, eye disease, hydrophobia, and chronic skin aliments. It is a potential herbal alternative that act as anti –cancer agent.(5)

II. MATERIAL AND METHOD

Collection- The rhizomes of curcuma longa,zingiber officinalis, the leaves of ocimum sanctum and fruits of solanum nigrum were collected from ahmednagar district,loni. The material was cleaned and dried at room temperature

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in shade ,away from direct sunlight and coarsely powder in grinder and powder material was passed through mesh to removed fine powder and coarse powder used for extraction..

Authentication- Mrs.priyanka ingle,scientist c botanical survey of india koregaon road,pune confirmation of plant,by compairing morphological features. The herbarium of the plant specimens has been deposited at B.S.I PUNE.
BSI/WRC/100-1/TECH./2019/33

EXTRACTION-

The fruit of solanum nigrum ,rhizome of curcuma longa ,zingiber officinalis,and leaves of ocimum sanctum,were collected and dried material is pulverized in grinder and this passed through 120 mesh sieves to remove fine powder and course powder used for extraction. This material extracted by two method soxhlet extraction method and maceration method.

Drugs and chemical-The following drugs and chemical were used.DPPH,PENTAZOCIN,CODEINE PHOSPHATE.

ANIMALS-

Experimental animal swiss albino mice(either sex)of 35-45g were acclimatized for 7 days under standard husbaandory condition ,i.e ,room temperature of (23±2)c,relative humidity of 45-55%and 0 light dark cycle of 12 :12 h.all the experimental protocols were approved by the institutional animal ethics committee take standard pellet food and water.all animal experiment were performed according to the committeefor the purpose of control and supervision of experiments on animal (CPCSEA) guideline.

Screening of syrup for antitussive, analgesic, antioxidant, antimicrobial action:

Using tail flick method (bhattacharya et al, 2014);

The tail flick examination was used to calculate analgesic activity by the method defined by D'amour and Smith 1941 [22], with minor alterations in the procedure. The tail flick method was utilized to study the antinociceptive activity in mice. A radiant heat automatic tail flick analgesiometer was applied to measure reaction latencies. Basal reaction time of animals to radiant heat was recorded by locating the tip (last 1-2 cm) of the tail on radiant heat source. The tail removal from the radiant warmth was taken as end point. The cutoff time of 15 seconds was used to avoid tail injury by heat. Mice were divided into five groups (). Mice were treated with morphine (10 mg/kg), normal saline, and Leonurus cardiaca (125, 250, and 500 mg/kg). The latent period of the tail-flick response was determined at 30, 45, 60, 75, and 90 minutes after the administration of drugs.

Scoring of Cough Bouts:

- Before administration of drug, the frequency of cough bout will be observed for all the animal groups at 0 min. It has been discussed that cough bout response to a given stimulus varies from animal to animal but fairly reproducible if repeat the measurements within the same animals.
- So, low or high cough bout threshold in animals were not entertained for further studies. The frequency of cough bouts will be observed for all animal groups at 1 hr after administration of standard drug, LPHF and MF by using same procedure and then percentage inhibition of frequency of cough bout will be calculated by the formula-

% percentage inhibition of frequency of cough = (1 – Ta / Ca) X 100
Where,
Ta= Frequency of Cough bout in tested herbal formulation treated animal
Ca= Frequency of Cough bout in control group treated animal

Table No.1:Formula for syrup

Part 1 Ingredients	**Weight**	**Function**
Paracetamol	2.5g	Active ingredient
PEG6000	10.0g	Solublizer
Glycerin	2.5g	Diluents &sweetener
D.M Water	30.0ml	Diluents

Anti-oxidant activity: **2,2 –Diphenyl -1-piceylhydrazyl (DPPH)assay:**

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The antioxidant activity of the extract were determine using the DPPH free radical scavenging assay described by nithianantham et al. and Zuraini et al. With some modifications. Briefly, the universal bottle was contained 50 ml of extract in concentration from 1to 5 mg/ml and 5 ml 0.004%(w/v)solution of DPPH was added. The obtain mixture was vortexed, incubated for 30 minute in room temperature in a relatively dark placed and then was read using spectrophotometer at 517nm.the blank was 80%(v/v)methanol. ascorbic acid was used for comparison. mesurement were taken in triplicate. DPPH scavenging effect was calculated using the following equation:
Where,
A0is the absorbance of negative control and A is the absorbance in presence of extract. the result were reported as IC_{50}values and ascorbic acid equivalents of extracts.

ANTIMICROBIAL ACTIVITY-

The antimicrobial assay was performed by agar well diffusion method. According tothis method, 0.1 ml of diluted inoculums (108 CFU/ml) of test organism was thoroughlymixed with 20 ml of molten sterile TSA and poured in pre sterilized petri dishes under sterilecondition. All plates were left to set at room temperature for 30-40 minutes. A well of 6mmdiameterwasmade in the centre of each seeded plates by using sterile cork borer. Holes werethen filled aseptically with 0.1 ml of Entoban syrup. Ciprofloxacin was used in comparison asa positive control. 1mg of ciprofloxacin was dissolved in 1ml of triple distilled water.Antibacterial plates were incubated at 37±10C for 24 hours. The antibacterial activity wasevaluated by measuring the zone of growth inhibition surrounding the well. The diameter ofinhibition zone was measured in millimeters (mm) by vernier caliper.

Antioxidant activity-

DPPH radical scavenging activity

The antioxidant activity was assessed by measurement of scavenging ability of the syrup and capsules on free radical 2,2'- diphenyl-1-picryl hydrazyl (DPPH; C18H12N5O6). Antiradical activity assay depends on the reduction of 1, 1-diphenyl-2-picrylhydrazyl. DPPH free radicals showed strong absorption maximum at 517 nm due to odd electrons. When this electron becomes paired off in the presence of a hydrogen donor for example any antioxidant, the absorption strength is decreased, and colour changed from purple to yellow, with respect to the number of electrons captured (Gülçin et al., 2005). 2, 2-Diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl (M.W= 394.24) (Sigma) was prepared in ethanol in the concentration of 3 mm. Each well in 96 well plate was labelled as control, blank and test compound of various concentrations. DPPH solution (95 µl) was added in the labelled wells. The test compound (5 µl) of M in DMSO) was then added in DPPHµconcentration 10- 1000 solution and reaction mixture was mixed for few seconds. The reaction was taken place in wells when 96 well plate was incubated at 37°C for 30 min. The micro titre plate was read at the absorbance of 515 nm (Spectramax plus 384 Molecular Device, USA) after 30 min. Percentage of radical scavenging activity was calculated with respect to DMSO treated control.

III. RESULT AND DISCUSSION-

Analgesic activity-

The tail lick test is test of the pain response in animal, similler to the hot plate test.it is used in basic pain research and to measure the effectiveness of analgesics,by observing the reaction of heat. Pretreatment with polyherbal syrup a (250 or 500 ml) demonstrated a significant and dose-dependent analgesic activity activity in the tail flick test . The 500 ml of polyherbal syrup a increased an analgesic activity in 30 (), 45, 60, and 75 () minutes after injection that were comparable to the normal saline. This effect was significant in time 45 and 60 minutes after injection for doses 125 and 250 mg/kg of polyherbal syrup. Under similar conditions, treatment with morphine significantly increased latency to thermal stimulation 30 min after administration and the analgesic effect was maintained during the entire period of evaluation.

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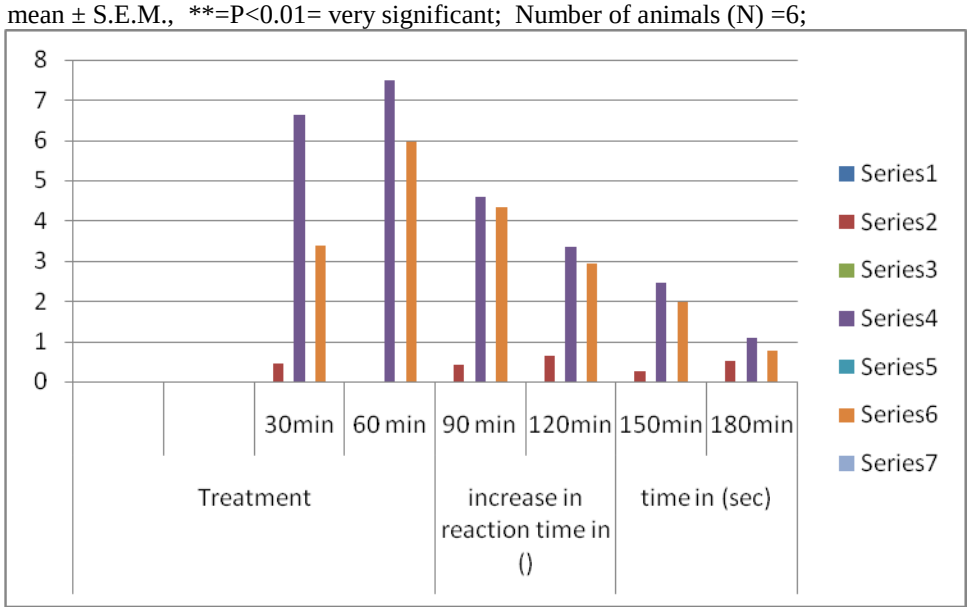
Table 1: Evaluation of analgesic activity by tail-flick method

Treatment	Increase in reaction time in (sec)					
	30 min	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
CONTROL	0.46 ± 0.34	0.01 ± 0.33	0.43 ± 0.73	0.66 ± 0.66	0.25 ± 0.66	0.53 ± 0.71
Standard	6.63 ± 0.62**	7.50 ± 0.28**	4.6 ± 0.52**	3.36 ± 0.24**	2.46 ± 0.29**	1.08 ± 0.35*
TEST	3.38 ± 0.78**	5.96 ± 0.54**	4.35 ± 0.50**	2.94 ± 0.46**	1.99 ± 0.31**	0.76 ± 0.22*

Results are mean ± S.E.M., **=P<0.01= very significant; Number of animals (N) =6;



Antitussive activity of polyherbal syrup-

The codeine phosphate (20 mg/kg, and 10 mg/kg, p.o.), a prototype cough suppressant, administered to animals produced 77.87%, 58.10% and 72.75%, 52.67% inhibition of frequency of cough bout induced by sulfur dioxide gas and Ammonium liquor after 60 min respectively. The extract of LPHF and MF in multiple doses showed dose-dependent cough suppressant activity, showed in Table 1 and Table 2. The extract of LPHF and MF (500 mg/kg, p.o.) exhibited significant activity i.e. 68.77% and 65.02% respectively, but lower dose (250 mg/kg, p.o.) of these showed less activity i.e. 37.30% and 34.19% respectively, inhibition in sulfur dioxide gas induced cough. While on the other hand, LPHF and MF extract (500 mg/kg, p.o.) exhibited significant activity i.e. 64.54% and 61.51% respectively, but lower dose (250 mg/kg, p.o.) of these showed less activity i.e. 34.02% and 28.65% respectively, inhibition in Ammonium liquor induced cough. All the anticough LPHF and MF formulations showed a significant inhibition in frequency of cough bouts albeit not to the extent shown by codeine phosphate

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Table 2-

Experimental group	Treatment	Dose (mg/kg)	No of Animals	Frequency of cough bout	percent inhibition of cough bout
Group I	control	Normal control	6	**85.34±1.47**	-
Group II	LPHF	**250**	6	58.33±1.51*	68.62
Group III	LPHF	**500**	6	31.83±1.19***	37.45
Group IV	MF	**250**	6	32.67±1.63*	70.20
Group V	MF	**500**	6	32.12±1.09**	37.79
Group VI	Codeine phosphate	**10**	6	39.33±1.11*	46.27
Group VII	Codeine phosphate	**20**	6	19.66±1.17***	23.13

values are mean ±SEM ,N=6, No of animal in each group

Control	Control	0
LPHF	LPHF	68.62
LPHF	LPHF	37.45
MF	MF	70.20
MF	MF	37.79
Codeine phosphate	Codeine phosphate	46.27
Codeine phosphate	Codeine phosphate	23.13

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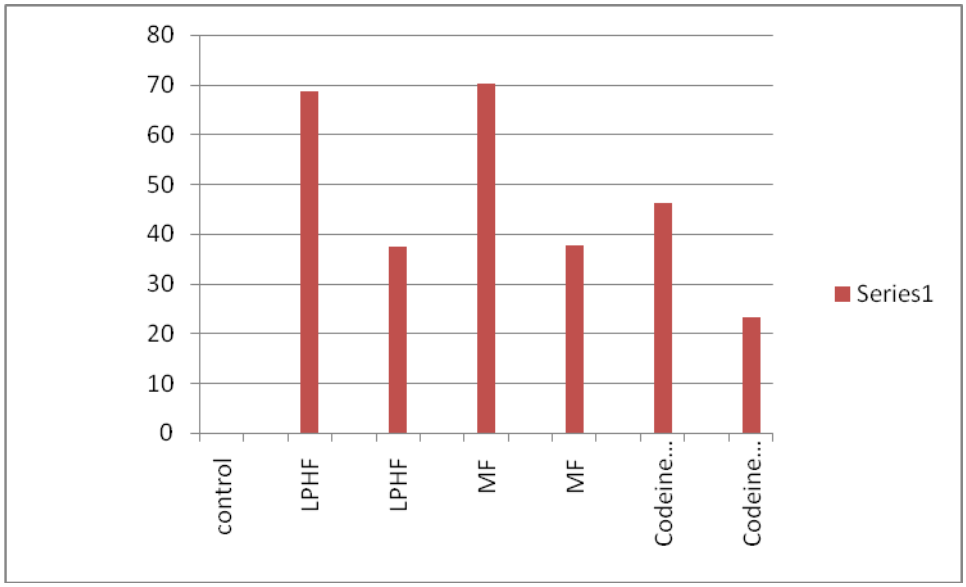

Antimicrobial activity of polyherbal syrup-

Polyherbal formulation was evaluated for its in vitro antimicrobial activity. Polyherbal syrup integrates an excellent combination of herbs . that have been used for decades to eliminate microorganisms and worms from gastrointestinal tract. It is the combination of Holarrhena antidysenterica, Berberis aristata,Symplocos racemosa, Querecus infectoria and Helicteres isora. Sucrose, a sweetening agent and main content of syrup, has been used to mask the bitter taste of the extract and so, a pleasantly tasting oral dosage form of the extract was formulated. Screening of anti-microbial activity was carried out by agar well diffusion method. An antimicrobial activity was evaluated against five gram negative bacterial cultures namely Salmonella enteric, Eschericia coli, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio cholera and one gram positive bacterial culture Staphylococcus aureus. The prepared syrup inhibited the growth of these organisms. It therefore indicates that the excipients and method of preparation did not affect the sensitivity of the active principles of the extract present in the formulation. Zone of inhibition of the developed formulation was comparable with the positive control.

Table 3

Test organism	Diameter of zone of inhibition(in mm)					
	1	2	3	4	mean±S.D(n=4)	positive control
gram positive bacteria						
staphylococcus aureus 19 17	18	19			18.25±0.957	23
gram negative bacteria						
salmonella enterica	24	21	21	22	22±1.438	26
eschericia coli	19	19	20	18	19±0.816	23
shigella dysenteriae	17	20	19	18	18.5±1.290	19
pseudomonas aeruginosa	20	17	17	19	18.25±1.5	24
vibiro cholerae	20	19	19	18	19±0.816	25

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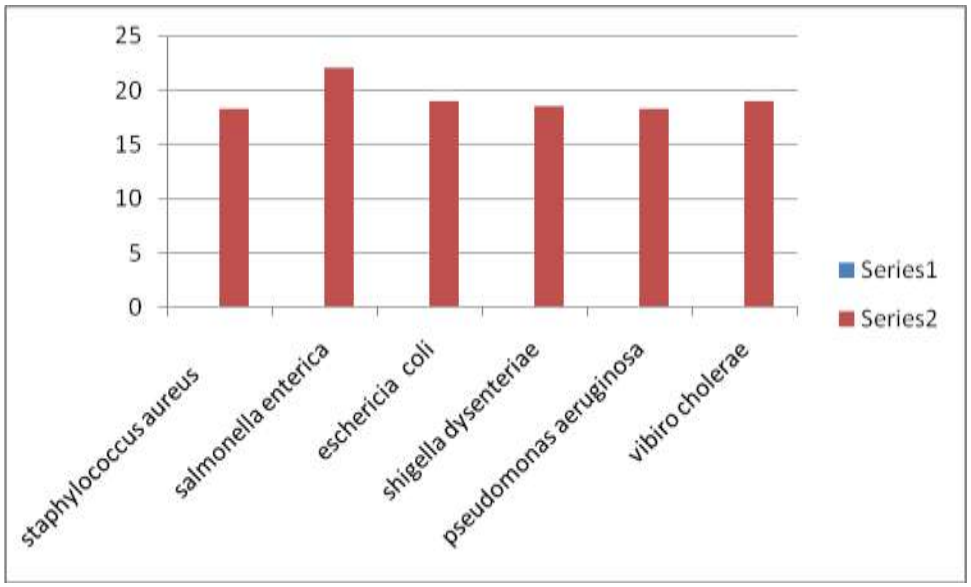
staphylococcus aureus	18.25
salmonella enterica	22
eschericia coli	19
shigella dysenteriae	18.5
pseudomonas aeruginosa	18.25
vibiro cholerae	19



Antioxidant activity of polyherbal syrup-

Excellent antioxidant potential with 8.5 and 10.3 µg/ml IC50 values respectively while BHA, the standard have 5.6 µg/ml IC50 value (Figure 1). When formulations of syrup and capsules were compared at various concentrations (10, 50 and 100 When formulations of syrup and capsules were compared at various concentrations (10, 50 and 100 µg/ml), DPPH radical scavenging activity increased in a dose dependent manner for both formulations just like standard BHA (Table 1). Results showed that both formulations of syrup and capsules have µg/ml), reducing ability increased in a dose dependent manner just like DPPH for both formulations just like standard BHA . Results showed that both formulations of syrup and capsules have goodreducing ability (Figure 2). Superoxide radicals are known to be very harmful to the cellular component. Super oxide free radical was formed by alkaline DMSO which reacts with NBT to produce colored diformazan. Formulations of syrup and capsules were compared at various concentrations. Both syrup and capsules have moderate activity to scavenge superoxide radicals

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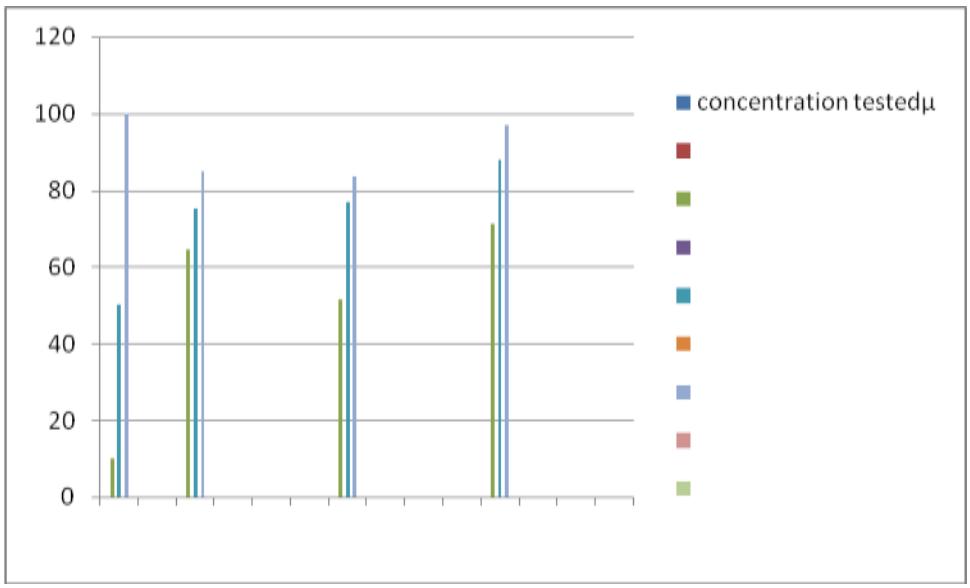
Table 4-

concentration testedμ μg/ml	percentage activity (syrup)±SEM	percent activity (capsule)±SEM	Percent activity(standard)±SEM
10	63.5±0.21	50.4±0.43	70.1±0.33
50	74.1±0.32	75.7±0.54	86.8±0.41
100	83.7±0.65	82.3±0.89	95.8±0.40



IV. CONCLUSION-

The use of Ayurvedic polyherbal formulation has stood the test of time.Using the Ayurvedic concept of panchmahabhutas and tridosha polyherbal formulations provide treatment of disease in a holistic approach.The scientific advancement carries with it the improvement of Ayurvedic formulationof polyherbal formulations through the study of various phytoconstituents and discovery of usefull herbs combination which produce desirable effect.Today the "renaissance''of Ayurvedic polyherbal formulation has occurred the plant over. Attributable to its comparable effectualness .Fewer sideeffect and higher satisfactoriness than medical aid drug most of the time they producesatisfactory impact and safety , making them one of the highly selected drug of choice.Nonetheless public inadequate knowledge and misconception on the safety of polyherbal formulation may results in the opposite effect such as toxicity undesired interaction.

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